

CLAIMS

1. A method of calibrating a liquid volume comprising:

providing a sample solution including a first chromophore having an absorbance
5 maximum at a first wavelength and a second chromophore having an absorbance maximum at a
second wavelength, the difference between the first and second absorbance maxima being at
least about 100 nm;

exposing the sample solution to electromagnetic radiation;

measuring an absorbance of the electromagnetic radiation by each chromophore;

10 exposing a blank solution to electromagnetic radiation, the blank solution being free of
the first chromophore and including the second chromophore in a concentration equal to that in
the sample solution;

measuring an absorbance of the blank solution; and

15 determining the volume of the sample solution, based on the measured absorbances of the
blank solution and the sample solution.

2. The method of claim 1, further comprising diluting the sample solution with a diluent.

3. The method of claim 2, wherein the diluent includes the second chromophore in a
20 concentration equal to that of the sample solution and is free of the first chromophore.

4. The method of claim 1, wherein the absorbance of the second chromophore at the first
wavelength is no more than about 10 % its absorbance at the second wavelength.

25 5. The method of claim 1, wherein the absorbance of the second chromophore at the first
wavelength is no more than about 5 % its absorbance at the second wavelength.

6. The method of claim 1, wherein the absorbance of the second chromophore at the first
wavelength is no more than about 2 % its absorbance at the second wavelength.

7. The method of claim 1, wherein the absorbance of the blank solution at the second wavelength is no more than 10 % its absorbance at the first wavelength.

8. The method of claim 1, wherein the second chromophore comprises ionic copper.

9. The method of claim 8, where the ionic copper is a soluble copper salt.

10. The method of claim 9, wherein the soluble copper salt comprises a compound selected from the group consisting of copper chloride and copper sulphate.

11. The method of claim 8, wherein the sample solution further includes a chelating agent for the ionic copper.

12. The method of claim 11, wherein the chelating agent comprises EDTA.

13. The method of claim 1, wherein the first chromophore is a compound selected from the group consisting of Amaranth, Ponceau S and Acid Red 1.

14. The method of claim 1, wherein the sample solution further includes a pH buffer.

15. The method of claim 14, wherein the buffer comprises a phthalate compound.

16. The method of claim 14, wherein the buffer has a concentration from about 0.005 M to about 0.05 M.

17. The method of claim 14, wherein the sample solution has a pH is from about 5.5 to about 7.

18. The method of claim 1, wherein at least one of the first and second chromophores has a temperature dependence of absorbance varying by no more than about 0.05 % per degree

centigrade.

19. The method of claim 1, wherein a plurality of sample solutions is provided, each having a unique concentration of the first chromophore, and wherein the plurality of sample solutions are provided in a multi-well plate.

20. The method of claim 19, wherein the determining step includes determining the volume of sample solution in each well.

21. The method of claim 19, further comprising measuring at least one dimension of each well of the multi-well plate to a level of accuracy of no more than 0.5 %.

22. The method of claim 19, further comprising measuring at least one dimension of each well of the multi-well plate to a level of accuracy of no more than 0.1 %.

23. The method of claim 21, wherein each well contains a sample solution having a unique concentration of the first chromophore.

24. The method of claim 1, wherein the sample solution is contained in a sample holder and the exposing step includes maintaining a contact angle from about 80 degrees to about 100 degrees between a meniscus of the sample solution and a wall of the sample holder.

25. The method of claim 24, wherein the maintaining step includes providing a salt in the sample solution in a concentration to achieve the desired contact angle.

26. The method of claim 24, wherein at least an interior portion of the wall is polystyrene.

27. The method of claim 24, wherein each well has a transparent bottom.

28. The method of claim 27, wherein the exposing step includes directing the

electromagnetic radiation through the transparent bottom.

29. The method of claim 1, further comprising utilizing computer-executable software for storing the measured absorbance.

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30. The method of claim 29, wherein the software calculates the volume from the measured absorbance.

31. A liquid volume calibration system comprising:

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a spectrophotometer for emitting and detecting electromagnetic radiation;
a multi-well plate, for containing a plurality of sample solutions and for exposing the solutions to the electromagnetic radiation;

each of the plurality of sample solutions including a first chromophore having an absorbance maximum at a first wavelength and a second chromophore having an absorbance maximum at a second wavelength, the difference between the first and second absorbance maxima being at least 100 nm, and each sample solution having a unique concentration of at least the first chromophore; and

a separate blank solution free of the first chromophore and including the second chromophore in a concentration equal to that in the sample solution.

32. A system comprising:

a plurality of sample solutions, each sample solution including a first chromophore having an absorbance maximum at a first wavelength and a second chromophore having an absorbance maximum at a second wavelength, the difference between the first and second absorbance maxima being at least 100 nm, and each sample solution having a unique concentration of at least the first chromophore;

a multi-well plate, for containing the plurality of sample solutions and for exposing the solutions to electromagnetic radiation, each well of the multi-well plate having a path length dimension to a level of accuracy of no more than about 0.5 %; and

a separate blank solution free of the first chromophore and including the second

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chromophore.

33. A system comprising:

a calibration plate for calibrating a first spectrophotometer with a second
5 spectrophotometer, the calibration plate having multiple cells containing a first set of calibration
solutions;

a second set of sample solutions each including a first chromophore having an
absorbance maximum at a first wavelength and a second chromophore having an absorbance
maximum at a second wavelength, the difference between the first and second absorbance
10 maxima being at least 100 nm;

a multi-well plate, for containing a plurality of the sample solutions for use in the first
spectrophotometer; and

a separate blank solution free of the first chromophore and including the second
chromophore in a concentration equal to that in the sample solution.

34. The system of claim 33, wherein each cell includes a gas for allowing expansion of the
sample solution, the gas being disposed in an area not exposed to the electromagnetic radiation.

35. A method of determining a liquid volume comprising:

providing a multi-well plate;

providing a sample solution having an unknown volume and contained in a well of the
multi-well plate;

maintaining a contact angle from about 80 to about 100 degrees between a meniscus of
the sample solution and the well, the contact angle being determined by concentrations of one or
25 more of a chromophore, a salt and a buffer in the sample solution;

exposing the sample solution to electromagnetic radiation;

measuring the absorbance of the chromophore; and

determining the volume of the solution, based on the measured absorbance and
concentration of the chromophore.

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Code for calculating a volume of a liquid sample solution based upon a photometric reading of absorbance, a concentration of a chromophore in the sample solution, a path length dimension of a sample holder in which the reaching is made, and a quantification of a non-linearity from the Beer-Lambert law of the reading.